

- SOP No:** AHP 75
- SUBJECT:** Serial cerebrospinal fluid (CSF) collection from intraventricular cannula (i.v.c.) in rats
- POLICY:** This procedure may only be performed by operators skilled in the technique.
Surgery must be performed under aseptic conditions
- PRECAUTIONS:** Surgical gloves, eye protection, long-sleeved gown, closed in shoes.
- All instruments and materials (including catheter) must be sterilized before use
- EQUIPMENT:** Rat with indwelling intraventricular cannula (i.v.c.), at least seven day postimplantation.
Absorbent-bench coat or 'Bluey'
Polyvinyl chloride (PVC) tubing (i.d. 0.28mm, o.d. 0.61mm, length 20cm, (available from Microtube Extrusions Pty Ltd, NSW, Australia)
Internal cannula for injection corresponding to the size of the indwelling cannula (28 gauge, i.d. 0.18mm, o.d. 0.36mm with 0.5ml projection (i.e. 9.5mm length), available from Plastics One, Aust.)
3ml syringes with attached (blunted) 26G needle (both sterile)
10µl glass Hamilton syringe
Distilled and micro-filtered water
500µl microfuge tubes; pre-labelled
Timer
Esky/cool-box containing dry ice
70% ethanol
Kidney dish and disposable Petri dishes
-80°C freezer
- PROCEDURE:**
1. Clean bench-top thoroughly with 70% ethanol
 2. Line the bench with bench-coat.
 3. Fill one Petri dish with 70% ethanol and one with filtered dH₂O and place on benchcoat
 4. Connect the internal needle to one end of the PVC line.
 5. Fill the 3ml syringe with filtered dH₂O.
 6. Connect 26G needle to other end of PVC line and flush the line with the dH₂O making sure no air bubbles exist in the line.
 7. Remove the 3ml syringe whilst holding both ends of the line together and upright (U-formation) thus not allowing the line to drain.

8. **Attach the Hamilton syringe filled with filtered dH₂O to the PVC line.**
9. **Evacuate the Hamilton syringe of dH₂O.**
10. **Draw the plunger back 2µl creating an air bubble at the needle-end of the line (this acts to separate the collected CSF from the dH₂O in the line.**
11. **Dip the internal needle into 70% ethanol followed by dH₂O and allow to air dry, not allowing it to touch any surface.**
12. **Obtain the animal with an implanted i.v.c. ([SOP AHP 74](#)).**
13. **Manually restrain animal during the procedure.**
14. **Clean the dummy cannula and surrounding cement cap thoroughly with a cotton tip soaked in 70% ethanol (NOTE; avoid getting it in the eyes of the rat).**
15. **Unscrew the dummy cannula closing the indwelling cannula and place it in the dish of 70% ethanol.**
16. **Gently clean the tip of indwelling cannula with the cotton tip.**
17. **Carefully introduce the internal needle to the indwelling cannula until it locks firmly into place.**
18. **Remove the dummy from the 70% ethanol and place it in the dH₂O.**
19. **Gently withdraw the plunger (approx. rate 4µl/min), CSF (clear, straw yellow and blood free) should be observed to travel up the line behind the air bubble.**
20. **Collect NO MORE than 6µl.**
21. **Remove the dummy from dH₂O and allow to briefly air dry.**
22. **Gently remove the internal needle from the cannula and replace the dummy**
23. **Screw it tight.**
24. **Return the rat to its box.**
25. **Set timer for next collection.**
26. **Make a note of the approx. volume of CSF collected and immediately evacuate the CSF from the line into a microfuge tube and immediately snap freeze on dry ice. Leave sample on ice until it can be stored in a -80°C freezer.**
27. **Remove the Hamilton syringe from the line.**
28. **Place the line in 70% ethanol and fill a 3ml syringe with 26G needle with 70% ethanol.**
29. **Flush the internal aspect of the line with 70% ethanol.**
30. **Fill a separate 3ml syringe with filtered dH₂O and flush the line again to remove ethanol.**
31. **Replace the dH₂O filled Hamilton syringe to the line and leave on the bench coat with the internal needle lying in the ethanol filled Petri dish.**
32. **Repeat 9-32 at the next time point.**
33. **Replace gloves between serial extractions.**

NOTE; steps 15-23 MUST be done efficiently to avoid exposure of the open cannula to the air as well as the risk of the collected CSF to evaporate. Extreme care and sterile technique MUST be taken during these procedures to avoid intracerebral infection. Wash all devices readily in 70% ethanol to minimise any such risk.

RECOMMENDATIONS:

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REVISED:

REFERENCES

1. Malhotra, B. K. Lemaire, M. and Sawchuk, R. *J.Pharm Res* (1994) 11, 1223.
2. Yang, Z. Huang, Y. Gan, G. and Sawchuk, R. J. (2005)*J Pharm Sci* 94, 1577.